

WHAT IS CLAIMED IS:

1 1. A method of using a functional cation channel protein in an assay for screening
2 potential drugs or agents which interact with the cation channel protein, the method
3 comprising the steps of:

4 a) providing a functional cation channel protein;

5 b) conjugating the functional cation channel protein to a solid phase resin;

6 c) contacting the potential drug or agent to the functional cation channel
7 protein conjugated to the solid phase resin;

8 d) removing the functional cation channel protein from the solid phase resin;
9 and

10 e) determining whether the potential drug or agent is bound to the cation
11 channel protein.

1 2. The method of Claim 1, wherein the providing step comprises:

2 a) expressing an isolated nucleic acid molecule encoding the cation channel
3 protein in a unicellular host such that the cation channel protein is present
4 in the cell membrane of the unicellular host;

5 b) lysing the unicellular host in a solubilizing solution so that the cation
6 channel protein is solubilized in the solution; and

7 c) extracting the cation channel protein from the solubilizing solution with a
8 detergent.

1 3. The method of Claim 2, wherein lysing the unicellular host in a solubilizing
2 solution comprises sonicating the unicellular host in a solution comprising 50 mM Tris
3 buffer, 100 mM KCl, 10 mM MgSO₄, 25 mg DNase 1, 250 mM sucrose, pepstatin,

4 leupeptin, and PMSF, pH 7.5.

5 4. The method of Claim 2, wherein the detergent comprises 40 mM decylmaltoside.

6 5. The method of Claim 1, wherein the conjugating step comprises binding the cation
7 channel protein to a cobalt resin at protein to resin ratio that allows for saturation of the
8 resin with the cation channel protein.

9 6. The method of Claim 1, wherein the removing step comprises contacting the cation
10 channel protein conjugated to the solid phase resin to an imidazole solution.

1 7. The method of Claim 1, wherein the isolated nucleic acid molecule encoding the
2 cation channel protein comprises a DNA sequence of SEQ ID NO:17, or degenerate
3 variants thereof, or an isolated nucleic acid molecule hybridizable under standard
4 hybridization conditions to an isolated nucleic acid molecule comprising a DNA sequence
5 of SEQ ID NO:17, or degenerate variants thereof.

1 8. The method of Claim 1, wherein the potential drug or agent is a member of a
2 library of compounds, and the contacting step comprises contacting the library of
3 compounds to the functional cation channel protein conjugated to the solid phase resin.

9. The method of Claim 8, wherein the library of compounds comprises a mixture of
compounds or a combinatorial library.

1 10. The method of Claim 9, wherein the combinatorial library comprises a phage
2 display library, or a synthetic peptide library.

1 11. A prokaryotic cation channel protein mutated to mimic a functional eukaryotic
2 cation channel protein.

12. The prokaryotic cation channel protein of Claim 11, selected from the group
consisting of a potassium channel protein, a sodium channel protein, or a calcium channel
protein.

1 13. The prokaryotic cation channel protein of Claim 11, endogenously produced in a
2 prokaryotic organism selected from the group consisting of *E.coli*, *Streptomyces lividans*,
3 *Clostridium acetobutylicum*, or *Staphylococcus aureus*.

1 14. The prokaryotic cation channel protein of Claim 11, comprising an amino acid
2 sequence of SEQ ID Nos: 1, 2, 3, or 7.

1 15. The prokaryotic cation channel protein of Claim 11, wherein said prokaryotic
2 cation channel protein is a potassium channel protein from *Streptomyces lividans*.

1 16. The prokaryotic cation channel of Claim 15, encoded by a nucleic acid comprising
2 a DNA sequence of SEQ ID NO:17, or degenerate variants thereof.

1 17. The prokaryotic cation channel protein of Claim 15, comprising an amino acid
2 sequence of SEQ ID NO:1, or conserved variants thereof.

1 18. The prokaryotic cation channel protein of Claim 11, wherein the functional
2 eukaryotic cation channel protein comprises a eukaryotic potassium channel protein, a
3 eukaryotic sodium channel protein, or a eukaryotic calcium channel protein.

1 19. The prokaryotic cation channel protein of Claim 11, wherein said functional
2 eukaryotic cation channel protein is endogenously produced in a eukaryotic organism
3 comprising insects or mammals.

1 20. The prokaryotic cation channel protein of Claim 19, wherein said eukaryotic
2 organism comprises *Drosophila melanogaster*, *Homo sapiens*, *C. elegans*, *Mus musculus*,
3 *Arabidopsis thaliana*, *paramecium tetraurelia* or *Rattus norvegicus*.

1 21. The prokaryotic cation channel protein of Claim 11, mutated to mimic a eukaryotic
2 cation channel protein comprising an amino acid sequence comprising SEQ ID Nos: 4, 5,
3 6, 8, 9, 10, 11, 12, 13, or 14.

1 22. The prokaryotic cation channel protein of Claim 21, wherein said prokaryotic
2 channel protein is a potassium channel protein from *Streptomyces lividans* comprising an

3 amino acid sequence of SEQ ID NO:1, said eukaryotic cation channel is a potassium
4 channel protein comprising an amino acid sequence of SEQ ID NO:4, and said mutated
5 prokaryotic channel protein comprises an amino acid sequence of SEQ ID NO:16, or
6 conserved variants thereof.

1 23. The prokaryotic cation channel protein of Claim 22, wherein said mutated
2 prokaryotic channel protein is encoded by an isolated nucleic acid molecule comprising a
3 DNA sequence of SEQ ID NO:17, or degenerate variants thereof.

1 24. An isolated nucleic acid molecule which encodes a mutant K^+ channel protein,
2 comprising a DNA sequence of SEQ ID NO:17, or degenerate variants thereof.

1 25. An isolated nucleic acid molecule hybridizable to the isolated nucleic acid molecule
2 of Claim 24 under standard hybridization conditions.

1 26. The isolated nucleic acid molecule of Claim 24, detectably labeled.

1 27. The isolated nucleic acid molecule of Claim 25, detectably labeled.

1 28. The detectably labeled isolated nucleic acid molecule of either of Claims 26 or 27,
2 wherein said detectable label comprises radioactive isotopes, compounds which fluoresce,
3 or enzymes.

1 29. The isolated nucleic acid molecule of either of Claims 24 or 25, which encode a
2 polypeptide comprising an amino acid sequence of SEQ ID NO:16, or conserved variants
3 thereof.

1 30. An isolated polypeptide comprising an amino acid sequence of SEQ ID NO:16, or
2 conserved variants thereof.

1 31. An antibody having a polypeptide of Claim 30 as an immunogen.

1 32. The antibody of Claim 31, wherein said antibody is a monoclonal antibody.

- 1 33. The antibody of Claim 32, wherein said antibody is a polyclonal antibody.
- 1 34. The antibody of Claim 33, wherein said antibody is a chimeric antibody.
- 1 35. The antibody of any of Claims 31-34 detectably labeled.
- 1 36. The antibody of Claim 35, wherein said detectable label comprises an enzyme, a
2 chemical which fluoresces, or a radioactive isotope.
- 1 37. A cloning vector comprising an isolated nucleic acid residue of either of Claims 24
2 or 25, and an origin of replication.
- 1 38. The cloning vector of Claim 37, wherein said cloning vector is selected from the
2 group consisting of *E. coli*, bacteriophages, plasmids, and pUC plasmid derivatives.
- 1 39. The cloning vector of Claim 37, wherein bacteriophages further comprise lambda
2 derivatives, plasmids further comprise pBR322 derivatives, and pUC plasmid derivatives
3 further comprise pGEX vectors, or pmal-c, pFLAG.
- 1 40. An expression vector comprising an isolated nucleic acid molecule of either of
2 Claims 24 or 25, operatively associated with a promoter.
- 1 41. The expression vector of Claim 40, wherein said promoter is selected from the group
2 consisting of the immediate early promoters of hCMV, early promoters of SV40, early
3 promoters of adenovirus, early promoters of vaccinia, early promoters of polyoma, late
4 promoters of SV40, late promoters of adenovirus, late promoters of vaccinia, late
5 promoters of polyoma, the *lac* the *trp* system, the *TAC* system, the *TRC* system, the major
6 operator and promoter regions of phage lambda, control regions of fd coat protein, 3-
7 phosphoglycerate kinase promoter, acid phosphatase promoter, and promoters of yeast α
8 mating factor.
- 1 42. A unicellular host transformed with an expression vector of Claim 40.
- 1 43. The unicellular host of Claim 42, wherein said host is selected from the group

2 consisting of *E. coli*, *Pseudomonas*, *Bacillus*, *Streptomyces*, yeast, CHO, R1.1, B-W, L-M,
3 COS1, COS7, BSC1, BSC40, BMT10 and Sf9 cells.

1 44. A method of producing a mutant cation channel protein comprising an amino acid
2 sequence of SEQ ID NO:16, or conserved variants thereof, comprising the steps of:

3 a) culturing a unicellular host of Claim 42 under conditions that provide for
4 expression of said mutant cation channel protein; and

5 b) recovering said mutant cation channel protein from said unicellular host.

1 45. A method of screening for compounds which selectively bind to a potassium ion
2 channel protein comprising:

3 (a) complexing a functional two-transmembrane-domain-type potassium ion channel
4 protein to a solid support;

5 (b) contacting the complexed protein/solid support with an aqueous solution said
6 solution containing a compound that is being screened for the ability to selectively
7 bind to the ion channel protein;

8 (c) determining whether the compound selectively binds to the ion channel protein
9 with the proviso that the potassium ion channel protein is in the form of a
10 tetrameric protein; and,

11 when the protein is mutated to correspond to the agitoxin2 docking site of a Shaker K⁺
12 channel protein by substituting amino acid residues permitting the mutated protein to bind
13 agitoxin2, the protein will bind agitoxin 2 while bound to the solid support, said
14 substituting of residues being within the 36 amino acid domain defined by -25 to +5 of the
15 selectivity filter where the 0 residue is either the phenylalanine or the tyrosine of the filter's
16 signature sequence selected from the group consisting of glycine-phenylalanine-glycine or
17 glycine-tyrosine-glycine.

1 46. A method of claim 45 wherein the solid supports are selected from the group

- 2 comprising: cobalt. insoluble polystyrene beads, PVDF, and polyethylene glycol.
- 1 47. A method of claim 45 wherein the two-transmembrane-domain-type ion channel
2 protein is a prokaryote.
- 1 48. A method of claim 45, wherein the two-transmembrane-domain-type ion channel
2 protein is from *Streptomyces lividans*.
- 1 49. A method of claim 45 wherein the two-transmembrane-domain-type ion channel
2 protein is KcsA.
- 1 50. A method of claim 45 wherein the two-transmembrane-domain-type ion channel
2 protein is mutated from a wild-type protein.
- 1 51. A method of claim 50 where the mutation is within the 36 amino acid domain
2 defined by -25 to +5 of the selectivity filter where the 0 residue is either the
3 phenylalanine or the tyrosine of the filter's signature sequence selected from the
4 group consisting of glycine-phenylalanine-glycine or glycine-tyrosine-glycine.
- 1 52. A method of claim 50 wherein the mutation deletes a subsequence of the native
2 amino acid sequence and replaces that the native with a subsequence from the
3 corresponding domain of a second and different ion channel protein.
- 1 53. A method of claim 52 wherein the second ion channel protein is from a eukaryote.
- 1 54. A method claim 45 wherein the aqueous solution comprises a non-ionic detergent.
- 1 55. A non-natural and functional two-transmembrane-domain-type potassium ion
2 channel protein wherein the non-natural protein is mutated in its amino acid
3 sequence from a corresponding natural protein whereby the mutation does not
4 prevent the non-natural protein from binding agitoxin2 when the non-natural
5 protein is further mutated to correspond to the agitoxin2 docking site of a Shaker
6 K⁺ channel protein said docking site created by substituting amino acid residues
7 selected from within the 36 amino acid domain defined by -25 to +5 of the Shaker

- 1 K⁺ selectivity filter where the 0 residue is either the phenylalanine or the tyrosine
2 of the filter's signature sequence selected from the group consisting of
3 glycine-phenylalanine-glycine or glycine-tyrosine-glycine.
- 1 56. A non-natural protein of claim 55 wherein the protein binds to a channel blocking
2 protein toxin with at least a 10 fold increase in affinity over the native ion channel.
- 1 57. A non-natural protein of claim 55 wherein the natural protein is the KcsA from
2 *Streptomyces lividans*.
- 1 58. A method of assessing the adequacy of the structural conformation of a
2 two-transmembrane-domain-type potassium ion channel protein for high through
3 put assays comprising the steps of:
- 4 (a) complexing a two-transmembrane-domain-type potassium ion channel protein
5 having a tetrameric form to a non-lipid solid support under aqueous conditions;
- 6 (b) contacting the complexed two-transmembrane-domain-type potassium ion channel
7 protein with a substance known to bind to the two-transmembrane-domain-type
8 potassium ion channel protein when bound to lipid membrane wherein the
9 substance also modulates potassium ion flow in that channel protein; and,
- 10 (c) detecting the binding of the substance to the complexed
11 two-transmembrane-domain-type potassium ion channel protein.
- 1 59. A method of claim 58 wherein the two-transmembrane-domain-type potassium ion
2 channel protein is mutated from a wild type two-transmembrane-domain-type
3 potassium ion channel protein by substitution of amino acids.
- 1 60. A method of claim 58 wherein the contacting is done in the presence of a non-ionic
2 detergent.
- 1 60. A method of claim 58 where in the substance is a channel blocker.

- 1 62. A method of claim 58 wherein the substance is a toxin.
- 1 63. A prescreening method for identifying potential modulators of potassium ion
2 channel function comprising:
- 3 (a) binding a soluble potassium ion channel protein to a solid support where the ion
4 channel has the scaffold of a two-transmembrane-domain-type potassium ion
5 channel and has a tetrameric confirmation;
- 6 (b) contacting the soluble potassium ion channel protein of step i with a compound in
7 an aqueous solution; and,
- 8 (c) determining the binding of the compound to the soluble potassium ion channel
9 protein.
- 1 64. A method of claim 63 wherein the contacting takes place in the presence of a
2 detergent.
- 1 65. A method of claim 63 wherein the ion channel can pass potassium ions when
2 expressed in a cell.
- 1 66. A method of claim 63 which further comprises the contacting of the compound to
2 cell expressing a two-transmembrane-domain-type potassium ion channel protein
3 said cell cultured in an aqueous media containing potassium and determining
4 modulation of potassium flow between the inside of the cell and the media.
- 1 67. A column comprising a solid support having bound thereto an ion channel having
2 the scaffold of a two-transmembrane-domain-type potassium ion channel and having
3 a tetrameric confirmation.
- 1 68. A column of claim 25 wherein the ion channel is a non-natural and functional
2 two-transmembrane-domain-type potassium ion channel protein wherein the

3 non-natural protein is mutated in its amino acid sequence from a corresponding
4 natural protein whereby the mutation does not prevent the non-natural protein from
5 binding agitoxin2 when the non-natural protein is further mutated to correspond to
6 the agitoxin2 docking site of a Shaker K⁺ channel protein said docking site created
7 by substituting amino acid residues selected from within the 36 amino acid domain
8 defined by -25 to +5 of the Shaker K⁺ selectivity filter where the 0 residue is
9 either the phenylalanine or the tyrosine of the filter's signature sequence selected
10 from the group consisting of glycine-phenylalanine-glycine or
11 glycine-tyrosine-glycine.